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ON THE EFFECTS OF SOLUTIONS OF VARIOUS ELECTROLYTES AND NON-CONDUCTORS UPON RIGOR MORTIS AND HEAT RIGOR.

BY

ANNE MOORE.

A DISSERTATION

SUBMITTED TO THE FACULTIES OF THE GRADUATE SCHOOLS OF ARTS, LITERATURE, AND SCIENCE, IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (DEPARTMENT OF PHYSIOLOGY).

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ON THE EFFECTS OF SOLUTIONS OF VARIOUS ELECTROLYTES AND NON-CONDUCTORS UPON RIGOR MORTIS AND HEAT RIGOR.

BY ANNE MOORE.

[*From the Hull Physiological Laboratory of the University of Chicago, Ill.*]

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INTRODUCTION.

SINCE the classic work of Kühne connected the occurrence of rigor mortis with the coagulation of proteids found in muscle plasma, the specific proteids present there have been subjected to repeated examination.[1] As a result of the adoption by Halliburton and

[1] HALLIBURTON, W. D.: Journal of physiology: 1884, v, p. 152; 1885, vi, p. 300; 1887, viii, p. 133; 1888, ix, p. 229; 1894, xv, p. 90; STEWART and SOLLMAN: Journal of physiology, 1899, xxiv, p. 427; v. FÜRTH, O.: Archiv für experimentelle Pathologie und Pharmakologie, 1895, xxxvi, p. 231; 1896, xxxvii, p. 389; Zeitschrift für physiologische Chemie, 1900, xxxi, 3 and 4, p. 338.

others of the method of fractional heat precipitation, which presupposes that each proteid has a definite coagulation temperature, the possibility of altering the supposed specific temperatures by the addition of salts of different kinds and concentrations to a solution containing the proteids in question has received more or less attention.

Although investigators do not agree as to the cause of coagulation,[1] it is generally accorded that the coagulation temperatures of the different proteids may be modified considerably by the presence of a salt and by the reaction of the solution.

The present work was undertaken at Dr. Loeb's suggestion and carried out under his direction to ascertain the effects of certain solutions upon (a) the temperature for the sudden coagulation of proteids in the muscle *in situ*, (b) the time necessary for coagulation at room temperature.

On the Effects of Solutions upon the Coagulation Temperature of Muscle Substance.

Method. — The gastrocnemius of the frog was used throughout the experiments. The muscle was placed in a covered dish containing 50 c.c. of the solution to be tested and was allowed to stand one hour at room temperature. It was then arranged for graphic registration upon the kymograph. A glass rod bent at right angles was screwed into the support holding the lever. The lower end of the rod drawn out to a fine point passed through the tendon of Achilles. The upper end of the muscle was attached to the short arm of the lever by means of a silk thread tied to a copper hook, which passed through the tendon covering the end of the femur. The solution was transferred to a beaker and arranged so that it surrounded the glass rod with it, attached muscle without touching the copper hook. A source of error pointed out by Vernon[2] was thus avoided. He found that if a muscle were attached by a thread which was wet by the solution

[1] Hardy regards coagulation as an affair of physical conditions due to the separation of the solid particles in a colloidal solution from the liquid. The influence of electrolytes which cause the solid particles to increase in size by a process of clumping he explains on an electrical basis. Journal of physiology, 1899, xxiv, p. 158. Pauli, on the other hand, explains coagulation on a chemical basis. Coagulation caused by the addition of a salt he thinks due to the formation of an insoluble double salt by the union of the proteid and the salt molecule. Archiv für die gesammte Physiologie, 1899, lxxviii, p. 315.

[2] Vernon, H. M., Journal of physiology, 1899, xxiv, p. 239.

variations in the length of the thread might easily be taken for muscular contraction. The lever magnified seven times; very small changes in the length of the muscle could, therefore, be determined. The beaker containing the solution was immersed in a water bath heated by a Bunsen burner. Half an hour was allowed for the temperature to rise from 15° C. to 65° C. A thermometer was fastened with its bulb as near as possible to the muscle and the rise in temperature was marked by hand upon the drum, which revolved once during an hour. When the muscle was first attached, the lever remained stationary if an equilibrium had been produced between the muscle and the solution, if not, slight changes in the level occurred, depending upon the changes which were being produced by the solution. When heat was first applied, this action of the lever continued until the critical temperature was reached, when it began to rise.

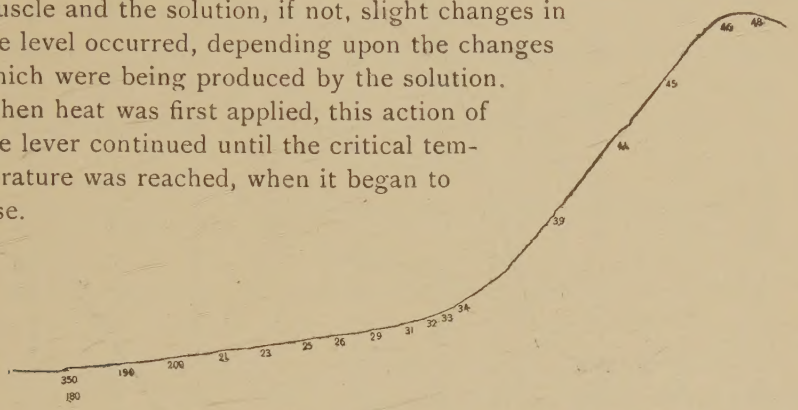

FIGURE 1. — Curve of heat rigor obtained in $\frac{n}{50}$ H_2SO_4.

The interpretation of curves. — The point at which the lever first began to rise varied from 15° to 41°. At about 47° it ceased to rise, at least for a time. In certain solutions it did not rise beyond this point (Fig. 1).

As a rule, after a short interval, a second rise began about 50° and continued to about 65° (Figs. 2 and 3). Occasionally a third elevation[1] appeared, but it was always slight when compared with the other two.

While the form of the curves is fairly constant in the same strengths of one solution, in differing solutions or in different strengths of the same solution much variation occurs.

In some solutions the rise was at first very gradual. This is interpreted as an indication of the stage described by workers on muscle plasma as the cloudy or opalescent stage which occurs before a defi-

[1] VERNON, working by the same method, also distinguishes three such contractions.

nite coagulum is formed.[1] Coagulation proper is marked by a sudden change in the angle of the curve. Figs. 2 and 3 serve as an illustration. In Fig. 3 coagulation occurs suddenly without a preliminary cloudy stage; in Fig. 2 the curve rises gradually, indicating a pronounced cloudy stage. At 33° it turns sharply, marking the occurrence of coag-

FIGURE 2. — Curve of heat rigor obtained in $\frac{n}{100}$ trichloracetic acid.

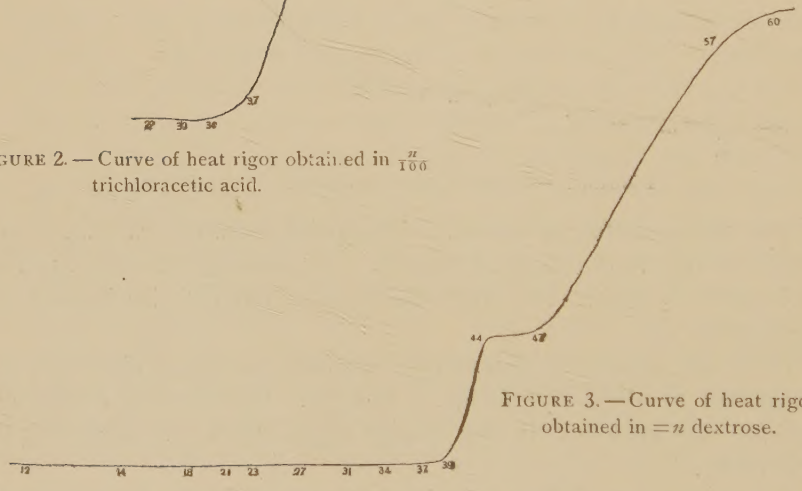

FIGURE 3. — Curve of heat rigor obtained in $= n$ dextrose.

ulation. A short time after the culminating point was reached the lever dropped suddenly. Strong salt solutions and solutions of acids and alkalies have a decidedly deleterious effect upon the tendon of Achilles, so that its connection with the muscle becomes impaired to such an extent that the two abruptly part as the temperature in-

[1] HAYCRAFT and DUGGAN, British medical journal, 1890, i, p. 167. CHITTEND and WYCKOFF-CUMMINS, Centralblatt für Physiologie, 1890, iii, p. 16. RINGER and SAINSBURY, Journal of physiology, 1890, xi, p. 369; 1891, xii, p. 170.

creases. In this case the gradual rise occurred in a very weak solution; it might be argued therefore that it indicates not opalescence, but merely absorption of water. This gradual rise is, however, not a distinguishing characteristic of weak solutions. In addition to this variation in the curve due to the occurrence or the suppression of the opalescent stage, other variations occur due chiefly to the relative

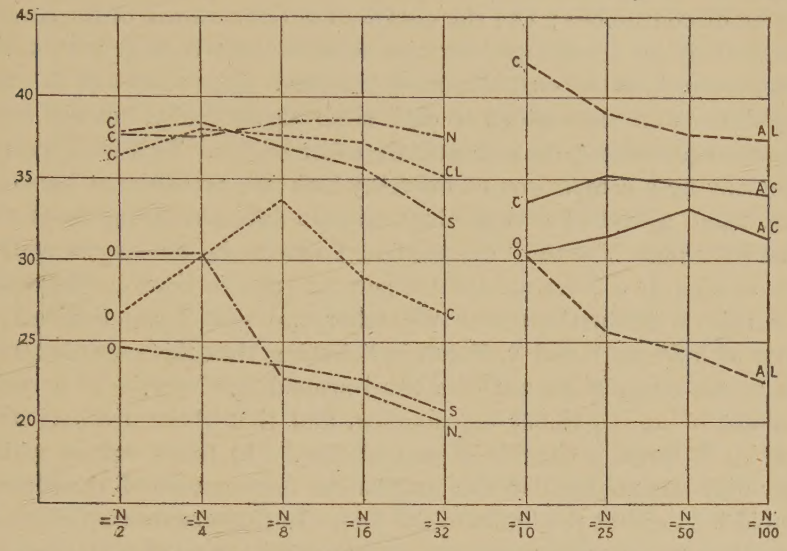

FIGURE 4. — Plotted curve showing average temperature of opalescence and coagulation in the various solutions. The temperature is indicated on the ordinate, the concentration on the abscissa.

C ——— AC = coagulation temperature of acids.
C - - - - - AL = coagulation temperature of alkalies.
C - - - - - - S = coagulation temperature of sulphates.
C . . . . . CL = coagulation temperature of chlorides.
C — - - — N = coagulation temperature of non-conductors.
O - - - - - S = opalescence temperature of sulphates.
O . . . . . C = opalescence temperature of chlorides.
O — - — - N = opalescence temperature of non-conductors.
O ——— AC = opalescence temperature of acids.
O - - - - - AL = opalescence temperature of alkalies.

height of the two contractions and to the interval separating them. All gradations were present between those curves which showed only one elevation and those which showed two. Where two elevations were present, they were sometimes approximately equal, sometimes the second was very slight in comparison with the first, and sometimes this relation was exactly reversed.[1]

[1] While the relative height of the sections of the curves cannot be taken as an accurate expression of the quantitative relations of the coagulated substances, at the

According to the accepted interpretation, each elevation in the curve corresponds to the coagulation of a definite proteid. If this is true, the variation in the number of elevations shown by my curves indicates a curious inconsistency. It is possible, however, that (1) a proteid may be so affected after a certain temperature is reached, that coagulation may be arrested for a time and then begin again, *i. e.*, it may be discontinuous; (2) the coagulation temperature of the second proteid may be so changed by the solution that it falls within the temperature limits of coagulation of the first. The results of the two coagulations are then added to each other algebraically, but the lever does not show where one ends and the other begins. This is suggested especially by a comparison of curves which are transitional between Figs. 1 and 2, and of curves obtained with different strengths of the same solution. The third contraction I find to be so very irregular, both as regards occurrence and temperature, and to be so insignificant as a rule in comparison with the other two that I am inclined to agree with Stewart and v. Fürth in believing that this contraction is due to the coagulation not of a third proteid but merely of a small remnant of uncoagulated myosinogen, and that there are only two proteids in muscle capable of coagulation.[1] In those curves which show only one contraction the coagulation temperature of the second proteid falls within the limits of the first. In those curves which show more than two contractions, the contraction of one or of both proteids has been made discontinuous. Hereafter these proteids will be referred to as the first and second proteid, the first coagulating at the lower temperature.

Results. — Pauli points out that the effects of salt solutions upon the coagulation temperature of proteids is a function of the physico-chemical peculiarities of both the salt solution and the colloid. They are not due merely to a general relation of colloids and crystalloids. He bases this opinion upon the fact that solutions of different salts

same time the comparison is interesting when taken in connection with the quantitative results of Stewart and v. Fürth. Stewart finds that the ratio of the amount of paramyosinogen to the amount of myosinogen present in muscles is 2.53, while v. Fürth finds that it is 0.3.

[1] Halliburton believes he has obtained four proteids from muscle plasma, paramyosinogen coagulating at 47° C., myosinogen at 56°, myoglobulin at 63°, muscle albumin at 73°, and myoalbumose not coagulable by heat.

Von Fürth and Stewart think that only two proteids have been isolated with certainty from muscle plasma, paramyosinogen, a typical globulin coagulable at 45°–50°, and myosinogen, an atypical globulin coagulating at 50°–65°.

having the same number of molecules in a given volume, or solutions of those which have the same degree of dissociation, do not affect similarly the coagulation temperature of egg-white, while two dilutions of the same salt may have the same effect upon the temperature. My results are in accord with this.

Pauli finds that an increase in the concentration of a dilute solution always causes an increase up to a certain point in the coagulation temperature. After the maximum point is reached the further addition of the salt causes no change, or an immediate fall in the coagulation temperature, or a gradual fall after a stationary period. Although I find this to be true in many solutions, I do not find it to be universal. In some dilute solutions, especially of acids, I found an actual decrease in the coagulation temperature with increasing strength; in other cases, the temperatures remained constant with increasing strength.

In general the entire height of the curve and also the height of the separate sections of the curve increase as the strength is decreased. This is true of sugar, urea, NaCl, LiCl, NH_4Cl, $CaCl_2$, $MgSO_4$, K_2SO_4 $(NH_4)_2SO_4$, NH_4OH, NaOH, KOH, LiOH, $KHSO_4$, $NaHSO_4$, tartaric, trichloracetic, acetic, oxalic, valerianic, and formic acids. In the strongest solutions coagulation may already have begun, leaving less substance to coagulate on being heated, but it is more probable that in dilute solutions an increase in the amount of H_2O present renders the coagulum more compact, so that it occupies less space. In certain of the solutions variations from this general rule occur. In $\frac{n}{10}$ $Ba(OH)_2$, for example, the first contraction is slight, the second very marked. As the strength is decreased, the first steadily increases in height as compared with the second. This is true also of $Sr(OH)_2$, KOH, and certain strengths of Na_2SO_4 and Li_2SO_4. In a few solutions, *e. g.*, HNO_3, H_2SO_4 and HCl, a decrease in the height of the curve appears as the strength is decreased. In NH_4Cl the height of the curve remains the same.

The effect of non-conductors. — If a perfectly fresh muscle be heated in a moist chamber, contraction will begin gradually at 29° and will continue to 47°, a sharp rise of the lever occurring at 37°. Later a second contraction begins at 51° and continues to 65°. After a muscle has remained in H_2O one hour at room temperature, the first contraction begins at 22° and continues to 46°, a sharp rise of the lever occurring at 32°; the second begins at 56° and continues to 62°. Considering the first contraction alone, which is all we shall attempt

to do in this paper, it seems that H_2O distinctly lowers both the temperature of opalescence and the coagulation temperature of the first proteid in muscle. This is what would naturally be expected, as coagulation occurs so readily in H_2O. Pauli, however, states that there is no simple relation between the coagulating power of a solution and the change in the coagulation temperature.

Pauli finds that urea and sugar have an effect similar to that of distilled water. This would naturally be true of a substance like urea where the molecules pass as freely in one direction as the other. The sugar molecule does not pass so readily, therefore somewhat different results might be expected. And, indeed, I find some difference in the action of the two substances. Glycerine and urea in all concentrations lower the temperature of opalescence. Weak solutions of dextrose and cane sugar also lower it, but strong solutions of dextrose and cane sugar raise it. Increase in concentration causes a sudden increase in the temperature of opalescence in urea, dextrose, and sugar, while the effect of glycerine is practically unaffected by the strength of the solution. The temperature of coagulation is slightly lowered by urea, and slightly raised by glycerine, dextrose, and sugar. A change of concentration in the various solutions produces very little effect on the temperature of coagulation.

The effect of electrolytes: Acids. — As a rule acids cause an increase in the temperature of opalescence. $\frac{n}{10}$ trichloracetic, $\frac{n}{10}$ tartaric, $\frac{n}{100}$ HCl and various strengths of HNO_3 lower it, however. In some of the acids, *e. g.*, valerianic, acetic, formic, H_2SO_4, very little difference results from a change in concentration; in others, however, there is a decided variation. In most cases, increase in concentration causes at first a slight increase in the opalescence temperature, and later a decided decrease. With the exception of certain strengths of acetic, trichloracetic, valerianic and formic acids, the temperature of coagulation was lowered by acids. The effect of increasing concentration upon the temperature of coagulation varied greatly. A comparison of the relative effects of acids shows that the change in temperature produced by them is largely dependent upon ionization. The greater the degree of dissociation of the acid, the more the temperature is lowered. At a dilution of 110, Dr. Loeb[1] gives the degree of dissociation of the acids used as follows:

[1] Loeb, J.: Archiv für die gesammte Physiologie, 1897, lxix, p. 1; 1898, lxxi, p. 457.

Valerianic . . .	0.04	H_2SO_4	0.80
Acetic	0.04	Oxalic	0.92
Formic	0.14	Trichloracetic . .	0.94
Malic	0.23	HCl	0.95
Tartaric	0.27	HNO_3	0.95

A comparison with Table II shows how closely this order is followed in the lowering of temperature. Apparently, therefore, the H-ion is responsible for the lowering of temperature. Dr. Loeb found, how-

TABLE I.

The temperature C. of opalescence (column *a*) and of coagulation (column *b*) after the muscle has remained in the given solution one hour at room temperature.

$= \frac{5}{4} n$[1]			$= n$			$= \frac{n}{2}$			$= \frac{n}{4}$		
	a	*b*		*a*	*b*		*a*	*b*		*a*	*b*
Cane sugar	40°	40°	Cane sugar	37°	38°	Dextrose .	38°	40°	Dextrose .	38°	39°
Urea . .	22°	33°	Dextrose .	36°	39°	Cane sugar	36°	37°	Cane sugar	36°	39°
			Urea . .	26°	35°	Urea . .	26°	36°	Urea . .	27°	35°
						Glycerine .	21°	38°	Glycerine .	21°	38°

$= \frac{n}{8}$			$= \frac{n}{16}$			$= \frac{n}{32}$		
	a	*b*		*a*	*b*		*a*	*b*
Dextrose . .	28°	40°	Dextrose . .	25°	?	Cane sugar .	21°	37°
Cane sugar .	22°	40°	Cane sugar .	23°	41°	Urea . . .	19°	38°
Glycerine . .	22°	38°	Glycerine . .	21°	38°			
Urea . . .	19°	36°	Urea . . .	19°	37°			

[1] The sign = is used here and throughout the paper to indicate solutions isosmotic with NaCl of the given strength.

ever, that the addition of H-ions to NaCl caused an increase in the taking up of water. This fact taken in connection with the power of H_2O to lower the coagulation temperature indicates that the effect of acids, though largely determined by the H-ion, may not be entirely independent of water absorption.

Alkalies. — The temperature of opalescence was much lowered by the alkalies used, except in the concentrations $\frac{n}{10}$, $\frac{n}{4}$, $\frac{n}{2}$, where it was raised. As the concentration is increased the temperature in-

creases until $\frac{n}{10}$ is reached, after which further increase in concentration causes a decrease in the temperature. The temperature of coagulation is raised by alkalies except in certain strengths of NaOH, KOH and $Ba(OH)_2$. Much variation in the temperature of coagulation was caused by increase in concentration. In order to further test the effects of acids and alkalies, 3 c.c. of $\frac{n}{5}$ NaOH and of $\frac{n}{5}$ HNO_3 were added to 100 c.c. $\frac{n}{8}$ NaCl and the results obtained in these solutions were compared with those obtained in $\frac{n}{8}$ NaCl. It was

TABLE II.

The temperature C. of opalescence (column *a*) and of coagulation (column *b*) after the muscle has remained in the given solution one hour at room temperature.

Acids.												
$2n$			n			$\frac{n}{2}$			$\frac{n}{4}$			
	a	*b*		*a*	*b*		*a*	*b*		*a*	*b*	
HNO_3	22°	27°	Acetic	36°	38°	Acetic	37°	37°	Acetic	37°	37°	
			H_2SO_4	31°	35°	H_2SO_4	30°	31°	H_2SO_4	31°	31°	
			HNO_3	28°	31°	HNO_3	26°	31°	HNO_3	25°	27°	
$\frac{n}{16}$			$\frac{n}{25}$			$\frac{n}{30}$			$\frac{n}{100}$			
	a	*b*		*a*	*b*		*a*	*b*		*a*	*b*	
Valerianic .	38°	42°	Valerianic .	37°	40°	Valerianic .	37°	39°	Valerianic .	36°	37°	
Acetic . . .	36°	39°	Acetic . .	36°	38°	Malic . . .	36°	37°	Trichloracetic	35°	35°	
Formic . .	34°	39°	Formic . .	35°	40°	Acetic . . .	35°	35°	Formic . .	34°	37°	
Malic . . .	33°	38°	Malic . . .	34°	37°	Formic . .	35°	36°	Oxalic . . .	34°	36°	
H_2SO_4 . .	30°	31°	Oxalic . . .	31°	33°	Oxalic . . .	34°	37°	Acetic . . .	33°	39°	
$NaHSO_4$ . .	30°	30°	Tartaric . .	30°	34°	Tartaric . .	34°	36°	HNO_3 . . .	32°	32°	
$KHSO_4$ . .	30°	30°	K_2SO_4 . . .	30°	32°	HCl . . .	33°	36°	$KHSO_4$ . .	31°	31°	
Oxalic . . .	29°	30°	$NaHSO_4$ . .	30°	30°	H_2SO_4 . .	32°	35°	$NaHSO_4$ . .	31°	31°	
HCl . . .	29°	31°	$KHSO_4$ . . .	30°	30°	Trichloracetic	32°	32°	H_2SO_4 . .	30°	32°	
Trichloracetic	27°	29°	Trichloracetic	29°	47°	$NaHSO_4$ . .	31°	31°	Malic . . .	29°	29°	
HNO_3 . . .	27°	27°	HCl . . .	29°	33°	$KHSO_4$ . .	31°	31°	Tartaric . .	29°	36°	
Tartaric . .	24°	36°	HNO_3 . . .	27°	28°	HNO_3 . . .	29°	32°	HCl . . .	24°	35°	

found that in the acid solution the temperature of both coagulation and opalescence was lowered; in the alkaline solution it was raised. The effects of alkalies upon temperature are thus seen to be exactly the opposite of those of acids. The effect of alkalies may be further shown by a comparison of the action of $\frac{n}{10}$ NaOH with that of the Na salts used isosmotic with $\frac{n}{8}$ NaCl. The OH ion elevates the coagulation temperature. This result indicates that the effects of the OH

TABLE III.

The temperature C. of opalescence (column *a*) and of coagulation (column *b*) after the muscle has remained in the given solution one hour at room temperature.

Alkalies.												
$2n$			n			$\frac{n}{2}$			$\frac{n}{4}$			
	a	*b*		*a*	*b*		*a*	*b*		*a*	*b*
NaOH	26°	27°	NaOH	30°	33°	NaOH	34°	37°	KOH	35°	38°
KOH	24°	26°	KOH	27°	30°	KOH	31°	33°	NaOH	32°	38°
$\frac{n}{10}$			$\frac{n}{25}$			$\frac{n}{50}$			$\frac{n}{100}$		
	a	*b*		*a*	*b*		*a*	*b*		*a*	*b*
$Sr(OH)_2$	39°	45°	KOH	29°	39°	LiOH	28°	39°	$Ba(OH)_2$	26°	38°
NaOH	37°	41°	LiOH	28°	39°	NaOH	28°	39°	NaOH	24°	32°
KOH	34°	40°	NaOH	27°	36°	$Ba(OH)_2$	25°	32°	LiOH	22°	39°
$Ba(OH)_2$	32°	44°	$Sr(OH)_2$	26°	39°	KOH	23°	41°	KOH	21°	?
LiOH	30°	41°	$Ba(OH)_2$	22°	38°	NH_4OH	22°	37°	$Sr(OH)_2$	21°	39°
			NH_4OH	22°	44°	$Sr(OH)_2$	20°	39°	NH_4OH	21°	39°

ion are specific effects independent of the entrance of water, for the presence of the OH ion as well as that of the H-ion causes increased absorption of H_2O.

Salts. — The chlorides as a rule lower the opalescence temperature, except when used in a strength isosmotic with $\frac{n}{8}$ NaCl, when the temperature is raised. $CaCl_2$ is a notable exception to this. It causes decided elevation of the temperature of opalescence. This is somewhat surprising as the tendency seems to be for good coagulants to lower the temperature. It seems to support the opinion of Pauli referred to above, p. 7. The difference may be due to the fact that

in Ca solutions little water is absorbed, while in solutions which lower the temperature much water is absorbed. With a few exceptions the temperature of coagulation is raised. The chlorides are good illustrations of Pauli's suggestion that osmosis is insufficient to account for

TABLE IV.

The temperature C. of opalescence (column *a*) and of coagulation (column *b*) after the muscle has remained in the given solution one hour at room temperature.

CHLORIDES.

$= \frac{5}{4} n$			$= n$			$= \frac{n}{2}$		
	a	*b*		*a*	*b*		*a*	*b*
NH_4Cl . .	43°	48°	NH_4Cl . .	38°	40°	$CaCl_2$ . . .	33°	37°
$MgCl_2$ . .	39°	41°	$MgCl_2$ . .	37°	38°	KCl . . .	29°	35°
$CaCl_2$ . . .	32°	40°	$CaCl_2$ . . .	34°	39°	NH_4Cl . .	27°	36°
KCl . . .	28°	54°	KCl . . .	25°	57°	$MgCl_2$ . .	24°	36°
NaCl . . .	?	41°	NaCl . . .	21°	58°	NaCl . . .	19°	39°

$= \frac{n}{4}$			$= \frac{n}{8}$			$= \frac{n}{16}$			$= \frac{n}{32}$		
	a	*b*		*a*	*b*		*a*	*b*		*a*	*b*
KCl	37°	39°	$MgCl_2$	36°	38°	$CaCl_2$	35°	40°	$CaCl_2$	33°	40
$CaCl_2$	35°	37°	NaCl	36°	38°	NaCl	33°	36°	NaCl	28°	35°
$MgCl_2$	31°	36°	LiCl	35°	37°	KCl	29°	36°	NH_4Cl	25°	30°
NH_4Cl	27°	38°	KCl	35°	39°	$MgCl_2$	27°	33°	$MgCl_2$	24°	32°
NaCl	21°	40°	$CaCl_2$	33°	37°	NH_4Cl	27°	39°	LiCl	22°	38°
			NH_4Cl	27°	38°	LiCl	22°	39°	KCl	21°	36°

coagulation phenomena. As concentration increases, increase of the opalescence temperature occurs until $= \frac{n}{8}$ is reached. Beyond this point, further increase in concentration causes first an increase, then a decrease in temperature.

With the exception of $MgSO_4$, in concentrations isosmotic with $\frac{n}{8}$ and $\frac{n}{4}$, NaCl, the sulphates decidedly lower the opalescence temperature. Weak solutions with the exception of $CaSO_4$ lower also the coagulation temperature. Strong solutions with the exception of certain strengths of K_2SO_4 raise it. Other Ca precipitating solutions

also lower the opalescence temperature, and with the exception of NaF they lower the coagulation temperature. As Ca is supposed to play so important a rôle in coagulation phenomena, it is interesting to compare these results with those obtained in $CaCl_2$. The opalescence

TABLE V.

The temperature C. of opalescence (column *a*) and of coagulation (column *b*) after the muscle has remained in the given solution one hour at room temperature.

SULPHATES.

$= \frac{5}{4} n$			$= n$			$= \frac{n}{2}$			$= \frac{n}{4}$		
	a	*b*		*a*	*b*		*a*	*b*		*a*	*b*
$MgSO_4$.	21°	39°	$MgSO_4$ . .	21°	39°	Na_2SO_4 .	29°	38°	$MgSO_4$ . .	31°	38°
Li_2SO_4 . .	21°	38°	Li_2SO_4 . .	21°	37°	$MgSO_4$.	26°	38°	Na_2SO_4 .	26°	42°
Na_2SO_4 .	21°	39°	K_2SO_4 . .	20°	33°	K_2SO_4 . .	25°	37°	K_2SO_4 . .	22°	36°
K_2SO_4 . .	19°	39°	Na_2SO_4 .	19°	38°	Li_2SO_4 . .	22°	39°	Li_2SO_4 . .	21°	38°
$(NH_4)_2SO_4$	17°	39°	$(NH_4)_2SO_4$	19°	38°	$(NH_4)_2SO_4$	21°	37°	$(NH_4)_2SO_4$	20°	39°

$= \frac{n}{8}$			$= \frac{n}{16}$			$= \frac{n}{32}$		
	a	*b*		*a*	*b*		*a*	*b*
$MgSO_4$ . . .	31°	37°	$MgSO_4$ . . .	27°	33°	$CaSO_4$ . . .	25°	40°
Li_2SO_4 . . .	23°	37°	$(NH_4)_2SO_4$.	23°	36°	K_2SO_4 . . .	22°	36°
K_2SO_4 . . .	22°	35°	K_2SO_4 . . .	22°	37°	$MgSO_4$ . . .	20°	27°
$(NH_4)_2SO_4$.	21°	37°	Li_2SO_4 . . .	22°	36°	Na_2SO_4 . .	20°	33°
Na_2SO_4 . .	20°	39°	Na_2SO_4 . .	20°	37°	$(NH_4)_2SO_4$.	19°	24°
						Li_2SO_4 . . .	18°	35°

OTHER CA-PRECIPITATING SOLUTIONS.

$= \frac{n}{2}$			$= \frac{n}{4}$			$= \frac{n}{8}$			$= \frac{n}{16}$		
	a	*b*		*a*	*b*		*a*	*b*		*a*	*b*
$Na_2C_2O_4$.	24°	35°	$Na_2C_2O_4$.	22°	35°	$Na_2C_2O_4$.	22°	32°	$Na_2C_2O_4$.	23°	34°
Na_2CO_3 .	21°	43°	Na_2CO_3 .	19°	?	NaF . . .	20°	56°	NaF . . .	22°	37°
NaF . .	19°	61°	NaF . . .	19°	56°	Na_2CO_3 .	19°	?	Na_2CO_3 .	21°	32°

temperature is raised in $CaCl_2$, lowered in Ca precipitating solutions. The coagulation temperature is little affected in $CaCl_2$; in most cases it is lowered in Ca precipitating solutions.

In determining the effect of external agents on heat rigor Vernon found that the temperature of onset of the initial heat contraction was lowered by hypotonic solutions and raised by hypertonic solutions. He thus makes the modification of the coagulation temperature of muscle proteids an affair of osmotic pressure. His opinion is based upon the use of NaCl alone. His experiments were too limited to allow the conclusion that osmosis is responsible for the results obtained. Undoubtedly the effects of osmosis are important. In those solutions in which much water is absorbed, there is a decided lowering in temperature, but in very strong solutions in which water is withdrawn the same results are often obtained. Osmosis therefore does not entirely explain the facts. In some cases the absorption of water seems to be a primary factor, and ionic effects secondary factors, merely governing the absorption of water, but the work of Hardy and Pauli has shown clearly the importance of electrolytes in coagulation phenomena.

On the Effect of Solutions upon the Time taken by Muscle Proteids to undergo Coagulation at Room Temperature.

Method. — The muscles were fixed in position as described above and left immersed in 100 c.c. of the fluid to be tested. The room temperature remained fairly constant at about 20° C. during the day; at night it was somewhat lower than this. The changes produced by the solutions were registered upon a drum which was turned by hand from time to time. The graphic representation of the muscular contraction was not to be depended upon as the sole test of coagulation, for contraction may be a specific effect of certain of the solutions. A heat test was therefore adopted. It is well known that normal muscles of the frog are thrown at once into rigor if they be heated to 40°, becoming white and opaque and firm to the touch. If a muscle is in rigor, sudden increase of the temperature to 40° causes no further contraction; if it is not in rigor, contraction occurs. After a muscle had remained in a solution for a certain length of time the solution was heated to 40°. If further contraction occurred, the muscle was evidently not in rigor; if no contraction occurred, there

were two possibilities, either coagulation of the first proteid at least had taken place, and the muscle was in rigor, or the solution had prevented the occurrence of rigor. The point was determined by the registration on the drum and the appearance of the muscle. Very often no contraction occurred at 40°, but on heating to 60° decided contraction occurred, showing that the coagulation of the second proteid takes place with much difficulty at room temperature, and suggesting that probably the second proteid is not necessarily concerned in normal rigor. The loss of irritability was used to indicate when to apply heat. So long as a muscle is irritable, rigor is not complete. The time of completion is determined by (1) the character of the solution, (2) its concentration, (3) its temperature. In those solutions in which rigor occurred in all concentrations it occurred more quickly in hypotonic and hypertonic solutions than in isosmotic solutions.

Results. — *The effect of non-conductors: Cane Sugar.* — In strong solutions the muscle becomes candied, *i. e.*, it is hard and transparent. No rigor can be said to be produced. In lower strengths rigor seems to occur. A slight contraction takes place and the muscle has the opaque appearance of natural rigor. In all the solutions except $= \frac{n}{32}$ rhythmical contractions occurred lasting from twenty minutes to an hour. It is quite probable that the sugar contained a small amount of Na, but it is not likely that the contractions were due to this. An impure solution of cane-sugar isosmotic with $\frac{n}{8}$ NaCl, would contain a very small amount of NaCl compared with a pure $\frac{n}{8}$ NaCl solution. The contractions in the sugar solution of that strength began at once, while in the pure $\frac{n}{8}$ NaCl they did not begin for an hour. These experiments were performed in the spring. The experiments upon which Dr. Loeb based his conclusions concerning rhythmical contractions were made in the autumn. It is a well-known fact that the spring frogs differ in their behavior from the autumn frogs. Whether this difference is indicative of a chemical difference which would account for such a phenomenon as rhythmical contractions in a non-conductor is yet to be determined. However this may be, it is not probable that the non-conductor as such is responsible for the contractions; it is more likely, as Howell[1] recently suggested, that the contractions are caused by the establishment of the necessary balanced proportion of ions as a result of a difference in the relative rapidity of diffusion of the dissociable ions.

[1] Howell, W. H.: This journal, 1901, vi, p. 181.

Dextrose. — In $= \frac{n}{2}$ dextrose contraction began at once and increased gradually. While it seemed to be a better coagulant than cane-sugar, no marked difference such as that noted by Locke[1] was noticed in its action. However, it was used in only two strengths.

Urea. — In all strengths rigor occurs. The height of the curve indicates that the coagulum is of a compact character. Remarkable absorption of H_2O occurs in urea. Its power to produce rigor may therefore be due to this quality. The marked similarity in the plotted time curve of urea and that of calcium was interesting. Rhythmical contractions were noted in strengths above $= \frac{n}{8}$. As in the case of sugar, the contractions began at once instead of after an interval as in $\frac{n}{8}$ NaCl. They could hardly be attributed therefore to the small amount of NaCl which might have been present in an impure solution.

Glycerine. — In $= \frac{n}{2}$ glycerine contraction began at once and continued gradually. After twenty-two hours no effect was produced on heating to either 40° or 60°. The effect of $= \frac{n}{16}$ was similar, the length of time, however, was increased to fifty-two hours.

The effect of salts: NaCl. — A strong solution causes a violent contraction of the muscle. This is followed by partial relaxation and twitching. When the twitching ceases, which occurs in a few moments, the lever retains its level. This contraction is not rigor and must be distinguished from it. The muscle remains irritable for some time and if suddenly heated to 40° contracts further. The rigor contraction may therefore be added to the first contraction algebraically. This fact is opposed to Hermann's idea that muscular contraction is a coagulation. The longer the muscle is left in the solution the less does it contract when the temperature is raised to 40° until finally no effect is produced by heating. By some sort of readjustment the first contraction seems to have passed over into rigor. The approach of rigor is not marked by further elevation of the lever, it may even be marked by a slight relaxation. Howell[2] notes a similar occurrence in oxalate solutions. If before rigor begins the muscle is changed to a weaker solution, *e. g.*, $\frac{n}{8}$ rapid relaxation takes place, *i. e.*, the first contraction is reversed and rigor may not occur. If rigor has begun but is not complete, the change does not interfere with further contraction but the process of completion will be slow. Water is withdrawn by the strong solution and re-enters from the weak solution. This in part explains the first case. In the second

[1] Locke, F. S.: Centralblatt für Physiologie, 1901, xiv, p. 670.

[2] Howell, W. H.: Journal of physiology, 1894, xvi, p. 476.

case, the entrance of water probably causes the formation of a more compact coagulum. If heat be applied some time after rigor is set, the lever may drop. This is not to be taken as evidence of reversibility, however, for the lowering of the lever may be due to various causes, *e. g.*, the giving up of water, the giving away of the tendon, or the solution of the clot. I have found no case of true reversibility after rigor is complete. Pauli finds that reversibility may occur in early stages in the heat coagulation of egg-white solutions, but it is impossible in later stages.

In weaker solutions, NaCl seems to lose the power of causing rigor. In $\frac{n}{2}$ the contraction is very slight, and solutions weaker than this cause no contraction except that which may take place in hypotonic solutions from the absorption of water.

KCl. — In strong solutions an immediate strong contraction with occasional twitching occurs. This is followed by a slight relaxation which sometimes amounts to one half the original contraction. Contraction then begins again and continues until rigor is complete. After rigor is complete relaxation again occurs. This is probably due to the dissolving of the coagulum. In weaker solutions the first strong contraction may not take place and the vigor with which the relaxation and further contraction take place is reduced. In $= \frac{n}{32}$, however, the rigor contraction is very marked, indicating a compact coagulum. The muscle appears more or less transparent, not firm and opaque as after heat rigor.

$CaCl_2$. — A strong solution has at first a relaxing effect, but after a period varying from half an hour to one hour and a quarter, contraction begins, the lever reaching its maximum height in from three quarters to four hours. If the solution is heated immediately to 40° further contraction takes place, showing that rigor is not completely set. Half an hour afterwards heat either produces no effect or causes a slight relaxation. In weaker solutions, $= \frac{n}{2}$, $= \frac{n}{4}$, contraction is slower in beginning and slower in setting, but as the strength of the solution is further decreased, rigor takes place more quickly.

In the case of blood and milk many authors suppose that Ca is essential to the formation of a clot. Starke asserts that no coagulation occurs without Ca. Hammarsten,[1] however, shows that coagulation occurs in the absence of all Ca salts precipitable by oxalates, and Duclaux[2] states that it is not proved that milk will not coagulate in

[1] Hammarsten, O.: A text-book of physiological chemistry.

[2] Duclaux: Traité de microbiologie, 1899, ii.

the absence of Ca. In the case of the muscle, Ca is possibly the most efficacious coagulant, but it is not essential to coagulation. Howell has shown this in his work on muscles treated with oxalate solutions.

$MgCl_2$. — The effect of $MgCl_2$ is relaxing. It not only does not cause rigor, but actually prevents it. A very slight contraction may take place at first. This is, however, followed by a very marked relaxation. Contrary to the above statement, however, rigor seems to be produced in the strength $=\frac{n}{2}$; the first relaxation is slight, lasting from three quarters of an hour to one hour and a half. This is followed by a decided contraction, lasting from five to twelve hours. After this no further contraction occurs on heating to 40°. Mg seems to have an especially deleterious effect upon the tendon. In strong solutions the tendon often breaks loose from the muscle on heating. After heat rigor has been produced in $MgCl_2$, its relaxing effect is still in evidence. On heating again contraction occurs. Apparently the coagulum is dissolved in $MgCl_2$ and is recoagulated on the application of heat. This could not be repeated more than once on the same muscle because putrefaction occurs in a few days.

Na_2SO_4. — As in the case of NaCl, strong solutions cause a marked initial contraction and a few rhythmical twitches. This is followed by relaxation lasting several hours, at the end of which contraction begins. This is the true rigor contraction. Unlike NaCl, it is separated from the initial contraction by a relaxation. The process is much slower than in NaCl. In isosmotic solutions the Na content is nearly the same; the difference is probably due to a difference between the action of SO_4 and Cl. In solutions weaker than $\frac{n}{2}$, rigor is not produced until $=\frac{n}{3\,2}$ is reached. Rigor caused in this strength is undoubtedly due to the absorption of H_2O.

$MgSO_4$. — No immediate effect was produced on the muscles immersed in the solution. A gradual rise began soon, however, indicating the approach of rigor, and the muscle assumed the usual rigor appearance. Rigor was noted in solutions stronger than $=\frac{n}{4}$. In $=\frac{n}{3\,2}$ it occurred as the result of water absorption. This result is in accord with that of Halliburton, who finds that strong and very weak solutions cause coagulation, while 5 per cent does not. According to J. R. Green [1] and Halliburton, both Na_2SO_4 and $MgSO_4$ when used alone restrain blood coagulation. Schäfer,[2] however, finds that $MgSO_4$ is a very good precipitant of serum globulin. In the case of muscle, I

[1] Green, J. R.: Journal of physiology, 1887, viii, p. 354.
[2] Schäfer, E. A.: *Ibid.*, 1880–82, iii, p. 181.

find that both are effective in certain strengths. When used together Na_2SO_4 and $MgSO_4$ are excellent precipitants of serum albumin. The difference between the action of $MgCl_2$ and $MgSO_4$ also indicates a difference in the action of the Cl and the SO_4 ion.

$\frac{n}{32}$ *CaSO*$_4$. — $CaSO_4$ is so insoluble that it could only be used in the strength $\frac{n}{32}$. Coagulation begins at once and proceeds gradually.

TABLE VI.

Time in hours after which no contraction occurred on raising the temperature to 40° C.

	$=5\frac{n}{4}$	$=n$	$=\frac{n}{2}$	$=\frac{n}{4}$	$=\frac{n}{8}$	$=\frac{n}{16}$	$=\frac{n}{32}$	H_2O
$CaCl_2$ . .	3	3	18	29	22	6	$5\frac{3}{4}$	22
NaCl . . .	1	$1\frac{1}{4}$	7 N	27 N	75 N	100 N	72 N	
$MgCl_2$ . .	3 N[1]	6 N	12	57 N	..	46 N	54 N	
KCl . . .	3	$3\frac{1}{4}$	24	30	70	36	47	
Na_2SO_4 . .	12	18 N	24	72 N	72	46	30	
$(NH_4)_2SO_4$	7	24	48	..	120 N	75	60	
$MgSO_4$ . .	12	16	16	18	40 N	50 N	40	
Cane sugar	60 N	22 N	22	18	30	40	48	
Urea . . .	8	9	28	30				
NaF . . .	..	..	4					
$Na_2C_2O_4$.	..	..	$5\frac{1}{2}$					
Na_2CO_3 . .	..	..	6					
Dextrose .	..	..	7	..	..	24		
Glycerine .	..	..	22	..	..	52		

	15°–20°	20°–25°	25°–27°	30°–34°	35°–37°	40°		
H_2O . . .	22	7–12	6	3	$1\frac{3}{4}$	22 min.		
$CaCl_2=n$.	..	..	2	1	..	$\frac{3}{4}$		
$NaF=\frac{n}{2}$.	..	..	..	$1\frac{1}{2}$				
$CaSO_4\ \frac{n}{32}$.	..	..	..	..	4			
$MgSO_4=5\frac{n}{4}$	..	..	..	..	$1\frac{1}{2}$			

[1] In the cases marked N no rigor occurred, or its occurrence was doubtful.

Coagulation would naturally occur in this strength as the result of water absorption. The marked contraction, however, indicates a specific Ca effect.

$(NH_4)_2SO_4$. — Coagulation occurs in all the concentrations used except $=\frac{n}{4}$ and $=\frac{n}{8}$. Pauli states that NH_4 compounds are not good coagulants, but Halliburton and v. Fürth have found them extremely effective. Rhythmical contractions are very pronounced in certain strengths.

NaF. — In $=\frac{n}{2}$ NaF immediate contraction occurs followed by a slight relaxation and an additional contraction. After from two hours and one half to four hours and one half no further contraction was produced, even on heating to 50°. NaF precipitates Ca. It would seem from this result that Ca is not essential to the coagulation of muscle proteids.

$Na_2C_2O_4$. — As is the case with NaF immediate contraction followed by relaxation and further contraction occurs in $=\frac{n}{2}$ $Na_2C_2O_4$. After five hours and one quarter no effect followed on heating to 40°. In one case a slight contraction occurred at the end of twenty-four hours on heating to 60°. Locke[1] states that muscle does not go into rigor after treatment with $Na_2C_2O_4$. Howell,[2] on the contrary, finds that oxalated muscle does go into rigor. From this he draws the conclusion that rigor is different from ordinary contraction, because contraction requires the presence of Ca while rigor does not.

Na_2CO_3. — In $=\frac{n}{2}$ Na_2CO_3 a contraction occurs followed by relaxation and further contraction. The effect is similar to that of NaF and $Na_2C_2O_4$; but the alkali influence appears in the height of the contraction, which is much more marked in this than in either of the other two solutions. After heating to 40° a slight relaxation is caused. Remarkable contraction occurs on raising the temperature to 60°.

Effect of acids and alkalies. — HCl, H_2SO_4, HNO_3, acetic, malic, tartaric, oxalic, valerianic, and trichloracetic acids were used in the strength $\frac{n}{10}$. *n* solutions were also used of H_2SO_4, HNO_3, and acetic acid. The immediate effect of all the acids was similar. They caused a sudden contraction of the muscle which jerked the lever magnifying fifteen times, to a height of four or five inches. Irritability lasted a half hour or more after this. The first contraction was followed at first by a slight relaxation, later by a slow, steady contraction. In HNO_3, H_2SO_4, and trichloracetic acid, the muscle assumed

[1] Locke, F. S.: Journal of physiology, 1894, xv, p. 119.

[2] Howell: *Loc. cit.*

every appearance of rigor. The heat test was, however, in every case followed by further contraction in $\frac{n}{2}$, $\frac{n}{10}$, $\frac{n}{50}$ H_2SO_4 at the end of two, five, sixteen, forty-seven hours, in $\frac{n}{10}$ HNO_3, forty hours. This result was somewhat puzzling. It was supposed that the outside of the muscle became coagulated, preventing the rapid penetration of the acid, and that the contraction was due to a sudden penetration of the acid to the inmost fibres of the muscle. It was found, however, that the contraction occurred in $\frac{n}{10}$ trichloracetic even after the muscle had been left in the solution three days. The only plausible explanation seemed to be that some one (or more) of the coagulated substances of the muscle was dissolved by the acid and was capable of recoagulating. And, indeed, it was found that the lever regained its level after a time. A muscle placed in trichloracetic acid and left three days was heated to 40°; the lever was raised four inches. The next morning it had sunk four inches. This process was repeated on three successive days. It was then supposed that a muscle thrown into heat rigor would be relaxed by acids. HNO_3, HCl, and trichloracetic acids were used, in each of which the process was as described above.

This is in accord with the experience of other observers. Hermann states that acids hasten rigor, but it is not complete, for myosin is dissolved. Stewart, Halliburton, and others also mention the power of acids to dissolve myosin.

The organic acids and HCl had a decided digesting effect upon the muscles. After being left in the solution for a time, the muscle assumed a transparent appearance and was easily macerated. In HNO_3, H_2SO_4, and trichloracetic acid, however, the appearance was entirely different. The coagulating effect of trichloracetic acid was at once evident and the acid soon reached the inside, rendering the muscle opaque and fibrous. In HNO_3 and H_2SO_4 the muscle was tough and resistant.

Hammarsten states that the additions of acids and alkalies retard the coagulation of blood, — the opposite of what I find in the case of muscle proteids. Halliburton, however, shows that in any coagulable fluid the greater the acidity up to a certain point the earlier coagulation occurs. In the case of milk this effect is well known. A certain similarity between the effects of acids and alkalies is noticeable. That the H and OH ions should have similar action is not surprising, for Loeb has shown that they act similarly in increasing the absorption of H_2O and in accelerating rhythmical contractions. As in the case

of acids immersion in an alkali is followed by a strong contraction in which the lever reaches greater height than in any of the neutral salt solutions, except possibly $CaSO_4$. Possibly a more compact coagulum is formed in acids and alkalies, or possibly, coagulation may be incomplete in salt solutions. Later relaxation occurs. The appearance of a muscle placed in an alkali is very different from its appearance when placed in HCl, H_2SO_4 or trichloracetic acid. It is more like that occurring in acetic acid. The muscle is more or less transparent and slimy. When it is heated, however, it retains its transparent quality and becomes decidedly elastic. It can be stretched, and will again resume its shape. $\frac{n}{10}$ NaOH, KOH, and $Sr(OH)_2$ were used. At the end of three hours and twenty-four hours it was found that heating to 40° produced no change. A strong contraction, however, occurred on heating to 60°. This indicates that coagulation of the first proteid had occurred, but not of the second. Both in the time and the temperature experiments the alkalies were found to exert a marked influence on the second proteid, the second contraction being both actually and comparatively much greater than in any other solution. Apparently some transformation occurs in this substance. This would accord with Starke's[1] idea. He believes that the dissolving and coagulating reactions of the globulins can only be explained on the supposition that the globulins exist in animal fluids only as alkali albumin compounds. The neutral salts are important in keeping them in solution, because they increase the alkalinity. In accord with Starke's idea that the neutral salts are of importance in increasing alkalinity it has been found that dialysis increases the acidity of muscle extracts and that after coagulation acidity increases.

A few experiments were tried in which acid and alkali in small quantities were added to various salts. The result was very striking in the case of $= n$ $CaCl_2$, and $\frac{n}{8}$ NaCl. The muscles from the same frog were put, the one into $= n$ $CaCl_2$, the other into 100 c.c. $= n$ $CaCl_2$ + 2 c.c. $\frac{n}{5}$ HNO_3. In the first, rigor was complete in three and one half hours; in the second a greater contraction occurred and at the end of one hour and a half no change took place on heating. In another experiment the muscles from a frog were put, the one into $= n$ $CaCl_2$, the other into 100 c.c. $= n$ $CaCl_2$ + 2 c.c. $\frac{n}{5}$ NaOH. In each contraction occurred almost at once. Four hours afterward the first contracted both at 40° and at 60°, the second did not contract at 40°, but contracted strongly at 60°. Similar results were obtained

[1] STARKE, J.: Zeitschrift für Biologie, 1900, x, pp. 419–446.

with NaCl. The solutions used were 100 c.c. $\frac{n}{8}$ NaCl + 3 c.c. $\frac{n}{5}$ HNO_3; 100 c.c. $\frac{n}{8}$ NaCl + 3 c.c. $\frac{n}{5}$ NaOH; 100 c.c. $\frac{n}{8}$ NaCl + 3 c.c. = $\frac{n}{8}$ cane sugar; $\frac{n}{8}$ NaCl. In the first two solutions an immediate contraction took place. At the end of two hours and three quarters no change occurred on heating to 40°. At 60° the tendon of the muscle in the acid solution gave way; the muscle in the alkaline solution contracted strongly. The muscles showed the characteristic acid and alkaline appearance. In the third and fourth solutions relaxation occurred. After thirty hours and three quarters contraction took place at both 40° and 60°.

If it has not been clearly proved that the formation of acid is responsible for both the onset and the solution of rigor as C. Schipiloff[1] has suggested, at any rate the increased formation of acid in fatigue is quite sufficient to account for the earlier onset of rigor in fatigued animals, for acids hasten the onset of rigor at ordinary temperatures and also lower the heat coagulation temperature. As acids and certain salt solutions have this effect there is no reason for adhering to the idea of a radical distinction between heat rigor and rigor mortis. They are both due in all probability to the coagulation in the muscle of substances corresponding to the proteids obtained from muscle plasma. In rigor mortis the coagulation temperature of muscle proteids has been lowered through the unwonted presence or juxtaposition of certain salts of H_2O or of acid. Either one or both proteids may be involved. The difficulty with which the coagulation of the second proteid occurs at room temperature suggests that this substance, either not at all or only in part, enters into the ordinary rigor mortis contraction.

Summary.

1. Stewart's and v. Fürth's conclusion that two proteids exist in muscle capable of coagulation receives support.

2. In general, hypotonic solutions, and other solutions in which much water is absorbed, lower the temperature of coagulation and shorten the time of completion of rigor.

3. Acids increase the temperature of opalescence and lower the temperature of coagulation, the amount of lowering increasing with the degree of dissociation.

[1] Schipiloff, C.: Centralblatt für die medicinischen Wissenschaften, 1882, p. 291.

4. Alkalies lower the temperature of opalescence and increase the temperature of coagulation. In alkalies a specific influence is present which may find its explanation in the formation of some such alkali compound as Starke suggests.

5. Rigor occurs readily in acids and alkalies at room temperature, but it is incomplete, for the coagulum tends to dissolve in these substances and also to some extent in some salt solutions. The dissolution is not a case of reversibility, for when once completely coagulated, the proteids of muscles do not resume their original character.

6. The chlorides in solutions isosmotic with $\frac{n}{8}$ NaCl show practical uniformity; the temperature of opalescence is raised, that of coagulation is only slightly affected. In other concentrations, variation occurs within wide limits; with the exception of $CaCl_2$, the temperature of opalescence in them is lowered, while the temperature of coagulation is lowered in weak solutions, raised in strong solutions.

7. Rigor occurs readily at room temperature in strong solutions of NaCl, Na_2SO_4, and $MgSO_4$, in $CaSO_4$, in all strengths of $CaCl_2$ and $(NH_4)_2SO_4$, and in other Ca precipitating solutions. It also occurs in KCl, but this substance is not a good coagulant. The effect of $MgCl_2$ is relaxing; rigor occurs only in $= \frac{n}{2}$ concentration.

8. Ca precipitating solutions affect the coagulation temperature somewhat differently from Ca solutions. Ca, however, is not essential for the coagulation of muscle proteids, unless the small amount retained in the tissues is sufficient, for coagulation takes place in Ca precipitating solutions and also in non-conductors.

9. Although the entrance of water favors coagulation and lowers the coagulation temperature, coagulation phenomena cannot be explained entirely on the basis of osmosis. For after a certain concentration is reached, further increase in concentration may cause either increase or decrease in temperature. The nature of the ions present is a determining factor.

10. Normal rigor is not comparable to ordinary muscular contraction, for (1) heat rigor may be added algebraically to contraction; (2) rigor is not reversible.

11. Heat rigor is not essentially different from normal rigor. Normal rigor is probably due to the lowering of the coagulation temperature of muscle proteids caused by the unwonted presence or juxtaposition in the tissue of acid, of H_2O, or of certain salts.